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# METHOD AND MEDICAMENT FOR PREVENTION OR MEDICATION OF HUMAN LEUCOCYTE ELASTASE-MEDIATED PULMONARY DISEASES

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## Abstract

A medicament and method for treatment of human leucocyte-mediated diseases comprising the administration of a polysulfated polysaccharide to the host.

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## Description

### METHOD AND MEDICAMENT FOR PREVENTION OR MEDICATION OF HUMAN LEUCOCYTE ELASTASE MEDIATED PULMONARY DISEASES

This invention was made with government support under Contract No. 5 RO1 HL 37615 awarded by the National Institutes of Health. The government has certain rights in this invention.

This invention relates to methods and pharmaceutically effective compounds useful in the prevention or mediation of human leucocyte elastase-mediated pulmonary diseases.

Human leucocyte elastase (HLE) (3.e.21.11) is a serine proteinase stored in the azurophil granules of polymorphonuclear leucocytes. HLE is capable of hydrolyzing most connective tissue components, especially elastin.

Destruction of elastin and the concomitant loss of elastic recoil in the lung parenchyma has been postulated as arising from an imbalance between elastase and its primary inhibitor, alpha-1-protease. The source of such possible imbalance is not known. The presumed role of elastase is to degrade bacterial cell wall components within the confines of the phagosome once the neutrophil has ingested a microbe.

Unfortunately, elastase is often released inadvertently into the nearby extracellular milieu during the process of neutrophil activation and microbial phagocytosis. To prevent injury by free elastase, alpha-1-protease binds to free elastase and inactivates it.

It has been suggested in the prior art that various pulmonary diseases are elastase-mediated. Emphysema, cystic fibrosis, acute respiratory distress syndrome, bronchial pneumonia, and other similar diseases are reported to be associated with excessive proteolysis, especially, elastolysis.

HLE exhibits rather unusual features when compared with other serine proteases such as trypsin, chymotrypsin and porcine pancreatic elastase. In the prior art, researchers have commonly used the more readily available chymotrypsin or porcine pancreatic elastase in studying the mechanism of catalysis of the serine family of proteases and their interaction with inhibitors. This has led to less than definitive conclusions, such as, for example, reported equivalent degradation of purified elastin by HLE and porcine pancreatic elastase, but with HLE exhibiting a preferential decrease in degradation of elastin contained in an extracellular matrix.

Several inhibitors, of a wide variety, have been developed for serine proteases, including irreversible inhibitors which are further classified as affinity-label inhibitors and mechanism-based inhibitors; and reversible inhibitors which are further classified as tight-binding, slow binding, slow tight-binding and classical. Affinity-label inhibitors have been tested with HLE but their high reactivity makes their use problematical. Mechanism-based inhibitors include haloenol lactones, chloropyrones, isocoumarins, and ynenol lactones. No in vivo studies are known to be reported for these compounds and their potential as therapeutic agents remains unknown. Whether one uses the affinity-labels or mechanism-based inhibitors, the goal is to achieve irreversible or at least long-term inhibition of the enzyme. This is precisely the mechanism for inhibition of elastase by alpha-1-protease inhibitor. This latter inhibitor, however, suffers from its susceptibility to oxidation which renders it ineffective as an elastase inhibitor. Thus, alpha-1-protease is rendered ineffective in the pulmonary tract by reason of the oxygen metabolites (H<sub>2</sub>O<sub>2</sub>), oxygen-derived free radicals (super oxide anion, hydroxyl radical), spin-altered states of oxygen (singlet oxygen), hypochlorous acid or oxidants associated with smoke inhalation (cigarette smoke).

Reversible inhibitors form a stable enzyme-inhibitor complex. Examples include peptide keto esters and peptide boronic acids. Whereas peptidyl boronic acid compounds have been found effective, in vitro, they are rapidly reversible and actually enhance HLE-induced emphysema in animal models.

Polysulfated polysaccharides, such as Arteparon (a sulfated glycosaminoglycan derivative of chondroitin sulfate), Structum (a polydisperse mixture of chondroitin-4-sulfate and chondroitin-6-sulfate, and disodium salt extracted from the cartilage of bovine trachea), and other compounds have been suggested as inhibitors of elastase in vitro (as the term is used in a general sense). However, Arteparon has been implicated in deaths from cerebral hemorrhage, thus rendering this compound and its

parent, chondroitin sulfate, seriously suspect for in vivo use. In similar manner, heparin has a small, but unfortunately serious risk of immune-mediated arterial thrombosis associated with its use so that this compound if useful for "in-hospital" therapy where its potential side effects may be carefully monitored, and therefore is not indicated as a practical outpatient pharmaceutical for elastase inhibition.

Moreover, the prior art teaches that the effectiveness of polysulfated polysaccharides, such as chondroitin sulfate (including Arteparon), heparin, and sulfated dextrans and chitosans are ineffective as elastase inhibitors at the lower molecular weight fractions of these compounds. The degree of sulfation of the compound is reported to strongly affect the inhibition efficacy of the compounds. For example, it has been reported that polysulfated dextrans having a molecular weight below about 100,000 Daltons are relatively ineffective as an elastase inhibitor when the degree of sulfation is less than about 1.

Contrary to the relatively voluminous teachings of the prior art that certain sulfated polysaccharides can serve as inhibitors of elastase in vitro, to the knowledge of the present inventors, there has been no successful development of an effective medicament for pulmonary tract-associated diseases that are mediated by HLE.

The problems associated with the prior art compounds and or techniques of administration of such compounds include such matters as noted hereinabove relating to the toxicity or undesirable side effects of certain polysulfated polysaccharides, ineffectiveness in the presence of oxidants, requirement that the compound be of relatively large molecular weight which complicates its delivery to sites within the pulmonary tract and which may contribute to its toxicity or the development of side effects. Other considerations when examining the inhibition of elastase in the pulmonary tract include solubility of the compound in aqueous media, electrolytic make-up of the compound (elastase is strongly cationic), physiological half-life, dispersibility in vivo, ability to target elastase, selectivity of reactivity, etc.

It is an object, therefore, of the present invention to provide a pharmaceutically acceptable medicament for HLE-mediated pulmonary diseases. It is another object to provide such a medicament which may be administered by aerosolization. It is another object to provide a novel method for the treatment or prevention of elastase-mediated pulmonary diseases.

The present invention comprises a medicament for the prevention or medication of human leucocyte elastase-mediated pulmonary disease comprising a treatment effective amount of a polysulfated polysaccharide.

In accordance with the present invention, the present inventors have discovered that through the oral application of pharmaceutically effective quantities of polysulfated polysaccharides (PS), as by the inhalation of a powder or liquid mist containing the compound, such PS effectively inhibit HLE-mediated diseases associated with the pulmonary tract. The pharmaceutically effective quantity is selected to be in the range of about 10 mg to about 100 mg q6h of the PS, based on the inhibitory activity of the medicament and the load of HLE in the lungs. The discovery that such relatively low dosages of the PS are effective permits the compound to be administered by aerosol, as opposed to intramuscular or intravenous administration which are recognized as potentially giving rise to adverse effects in vivo. Further, such discovery has provided the means for limiting the exposure of the body to the potentially damaging side effects of certain of the PS compounds which do appear to be partially a consequence of, or at least exacerbated by, the prior art requirement that such compounds be administered in substantially sized dosages in order for them to be effective. Still further, the PS of the present invention have been found to effectively seek out the alveolar interstices and remain available for half-life time periods that permit the administration of the compound in amounts and at times that are reasonable and acceptable for patients. To this end, the present compounds have been found effective when administered orally as by inhalation at intervals of up to about eight hours. The sulfated polysaccharides of the present invention are "naturally occurring" and commonly are present in the extracellular matrix. The fact that sulfated polysaccharides of the present invention are normal constituents of lung matrix indicates that these compounds provide a tissue based protection of elastin from HLE-mediated proteolysis, thereby differentiating the present compounds from other compounds known to provide in vivo inhibition of elastolytic activity.

The compounds of the present invention are reversible as regards their inhibitory effect upon elastase. That is, the elastase is inhibited as by the electrostatic binding of the PS to the opposite ends of the elastase molecule, leaving the effective center portion of the elastase molecule unaltered. Importantly, the compounds of the present invention remain extracellular so that they are available to inhibit extracellular elastase without disruption of the desirable and beneficial function of intracellular elastase, which in its

"natural" and effective state is a host defense against bacteria. Further, the PS compounds of the present invention are not subject to being rendered ineffective by oxidants in the pulmonary tract, they are not adversely affected by normal pH fluctuations in the tract, and they are selective in that they do not attack the parenchyma tissue nor inhibit the normal functioning of other desirable metabolic activities, including desirable proteolytic activities. The present PS compounds have been found to not induce adverse hemorrhaging, and in fact have been noted to effect a reduction in established hemorrhaging due to elastase-induced diseases in the pulmonary tract.

The present PS compounds, when administered in accordance with the present disclosure are considered effective in the treatment of any HLE-mediated disease of the pulmonary tract. For example, it is known that the bacteria, *Pseudomonas aeruginosa*, is commonly present in the pulmonary passages of normal persons but such persons do not contract pneumonia. However, up to two percent of the patients entering hospitals contract pneumonia and of these patients, 50% die from such hospital-contacted pneumonia.

The present PS compounds, inhaled routinely in the course of a hospital stay, are deemed to provide prophylactic protection against such pneumonia, most likely by reason of the fact that the present PS compounds inhibit the development of colonization sites for the bacteria by the proteolytic activity of elastase in the pulmonary tract.

FIGURE 1 is a graphical representation of data indicating the inhibition of HLE by sulfated polysaccharides. The substrate used was suc-ala2-val-pNA.

FIGURES 2A, 2B and 2C are graphical representations of data showing the effect of GAGPS on acute lung injury induced by HLE. FIGURE 2A depicts the hemoglobin/ml lavage, FIGURE 2B depicts the protein/ml lavage, and FIGURE 2C depicts the granulocyte count/ml lavage.

FIGURES 3A, 3B and 3C are graphical representations depicting the effect of GAGPS on HLE-mediated emphysema as indicated by quantification of the alveolar intercepts in randomly selected fields for each lung examined. In FIGURE 3A, 6K GAGPS was administered four hours after the HLE; in FIGURE 3B, 10K GAGPS was administered simultaneously with the HLE; and, in FIGURE 3C, 19K GAGPS was administered four hours after the HLE. In all instances, the extent emphysema was reduced after addition of the GAGPS and are maintained reduced for up to eight hours.

FIGURE 4 is a graphical representation depicting the effect of dextran sulfate on acute lung injury induced by HLE as represented by the analysis of protein in lavage.

FIGURE 5 is a graphical representation depicting the effect of dextran sulfate on acute lung injury induced by HLE as represented by the analysis of hemoglobin in lavage.

In accordance with the present invention, the preferred inhibitor is dextran sulfate having the following formula:

where:

$n = .SO_3^-, H$

R1, R2 and R3 each = a lower alkyl

This compound is a derivative of dextran, or polyglucose composed of alpha-D-glucopyranosyl units linked predominantly alpha-D(1-6) and differing only in chain length as defined in The Merck Index, 10th Ed., 1983; 426.

Dextran is produced by bacteria growing on a sucrose substrate. A number of methods of production exist, including methods of removing pyrogens, and for producing clinical dextran (U.S. Patent Nos. 2,762,727 and 2,972,567). Dextran sulfate is prepared by boiling dextran with sulfuric acid to reduce the molecular weight and esterifying the degraded dextran with chlorosulfonic acid in pyridine as described in U.S. Patent No. 2,715,091, which patent is incorporated herein by reference.

The activity of dextran sulfate was tested by monitoring the ability of HLE purified from an extract of polymorphonuclear leukocytes using Matrex Gel Orange A chromatography followed by cation exchange chromatography on Bio-Rex 70 as described by Kao et al, Proteinase 3: A Distinct Human Polymorphonuclear Leukocyte Proteinase That Produces Emphysema in Hamsters, J. Clin Invest 1988; 82:1963-1973 to cleave the synthetic elastase

substrate succinyl-tri-alanyl-p-nitroanilide, causing an increase in spectrophotometric optical density at 405 nm. The reaction was run in 0.125 M HEPES buffer (pH 7.5) containing 0.125% Triton X-100, 3 mM substrate, 20 ssg/ml of HLE and various molar ratios of elastase to inhibitor. Various molecular weight fractions of dextran sulfate were tested, the results being depicted in Table I.

TABLE I  
EFFECT OF DEXTRAN SULFATE  
AND DEXTRAN ON HLE  
Ratio of Elastase O.D. Change % Inhibition  
to Inhibitor at 405 nm -----

A. Dextran Sulfate

(mol. wt. 5000)

1:0 0.085 0  
1:0.02 0.081 5  
1:0.2 0.066 29  
1:0.4 0.045 47  
1:0.5 0.040 53  
1:1 0.014 84  
1:2 0.010 88  
1:4 0.004 95

B. Dextran Sulfate

(mol. wt. 8000)

1:0 0.085 0  
1:0.02 0.088 20  
1:0.2 0.082 27  
1:0.4 0.043 49  
1:0.5 0.032 62  
1:1 0.011 77  
1:2 0.007 82  
1:4 0.004 95

C. Dextran

(mol. wt. 9000)

1:0 0.099 0  
1:0.1 0.099 0  
1:0.2 0.099 0  
1:0.4 0.113 0  
1:0.5 0.111 0  
1:1 0.112 0  
1:2 0.112 0

As shown in Table I, dextran alone provides no inhibitory activity toward HLE. On the other hand, the inhibitory activity of dextran sulfate is almost complete at a ratio of 1:2 elastase to inhibitor. Such activity is thus a function of the molecular weight of the dextran sulfate and its degree of sulfation.

Chondroitin sulfate having a molecular weight of 55,000 Daltons (purchased from Sigma Chemical of St.

Louis, MO.) and heparan sulfate having a molecular weight of 40,000 Daltons purified from EHS sarcoma as described by Hassell et al, Isolation of a Heparan-sulfate Containing Proteoglycan From Basement Membrane, Proc. Natl. Acad.

Sci. USA, 1980; 77:4494-4498, were tested for inhibitory activity in like manner to the testing of dextran sulfate.

The results of such tests are given in Tables II and III.

TABLE II  
EFFECT OF CHONDROITIN SULFATE  
ON HLE

Ratio of Elastase O.D. Change % Inhibition to Inhibitor at 405 nm -----

1:0 0.080 0  
1:0.1 0.018 77  
1:0.2 0.014 82  
1:0.4 0.014 82

1:0.5 0.015 81

1:1 0.016 81

TABLE III

# EFFECT OF HEPARAN SULFATE

ON HLE

Ratio of Elastase O.D. Change % Inhibition to Inhibitor at 405 nm -----

1:0 0.096 0

1:0.1 0.083 14

1:0.2 0.052 46

1:0.4 0.029 70

1:0.5 0.024 75

1:1 0.017 82

Both chondroitin sulfate and heparan sulfate inhibited elastase in vitro almost completely at a 1:1 ratio of inhibitor to elastase.

In further testing, the activity of HLE was monitored using the specific synthetic chromogenic substrate suc-ala2-val-pNA. The method used is described by Barrett, A. J., Cathepsin G, Methods Enzymol. 1981; 80: 561-565 with modifications. The assay mixture of 1 ml contained 0.3 mM substrate (100 1 3 mM in DMSO) in 50 m HEPES buffer, pH 7.5. The reaction was started by addition of 100 p1 of HLE (20 ssg/ml). Activity against the substrate was determined by release of 4-nitroaniline as indicated by an increase in OD at 405 nm over 3 minutes.

Inhibition was assessed by preincubation of HLE with sulfated polysaccharides for 30 min at 37°C prior to initiating the reaction. Tests were performed employing dextran sulfate, heparin, heparan sulfate, chondroitin sulfate, dextran sulfate and dextran. The results of these tests are presented in Figure 1 wherein it is noted that each of these additional compounds in a concentration of less than 400 ng/ml is an effective inhibitor of HLE.

The activity of HLE itself was also assessed with insoluble elastin as the substrate. Bovine ligament elastin was prepared by the method of Starcher and Gallone (Starcher et al, Purification and Comparison of Elastins from Different Animal Species, Anal Biochem 1976; 74:411-447) and assessed for purity by amino acid analysis. Its degradation was assayed using elastin radiolabelled with [3H] NaBH4 following the methods described by Stone et al, Proteolysis of Insoluble Elastin, Methods Enzymol 1982; 82:588-605. The tritiated powdered elastin was homogenized and washed in PBS, pH 7.4. The reaction mixture containing the reference enzyme or sample preincubated with inhibitor was added to a 5 mg aliquot of 3H-elastin and incubated at 37°C, pH 7.4. Solubilized peptides were separated from the elastin suspension by filtration through medium porosity filter paper. The rate of degradation was determined by quantifying the 3H-peptides solubilized. Table IV presents the results of these tests and indicates that GAGPS of molecular weights between about 6,000 and 19,000 Daltons effectively reduces the HLE activity at molar ratios of HLE to GAGPS as low as about 1:0.2 at the higher molecular weights while requiring higher ratios, i.e. above about 1:0.5 at the lower molecular weights.

TABLE IV

HLE ACTIVITY AGAINST

3H-ELASTIN IN PRESENCE OF GAGPS

GAGPS

Molar Ratios 2.8K 6K 10K 19

HLE:GAGPS

1:0 100 100 100 100

1:0.1 136 113 87 58

1:0.2 95 84 48 17

1:0.4 127 81 19 31

1:0.5 108 56 - 14

1:1 118 25 - 18

The ability of polysulfated polysaccharides to prevent HLE-mediated acute lung injury was assessed in female Syrian golden hamsters (Harlan Industries, Indianapolis, IN.) weighing 90-110 g. Pentobarbital anesthetized hamsters were injected intratracheally with 0.25 ml sterile 0.9% saline (NS) or 0.25 ml NS containing fractions of Arteparon (GAGPS), a supersulfated derivative of chondroitin sulfate, having respective molecular weights of 2,800, 10,000 and 19,000 Daltons and with a limited degree of polydispersity. These injections of GAGPS were followed at timed intervals by injection of HLE in 0.25 ml

NS. Twenty-four hours after the treatment, anesthetized animals were sacrificed by exsanguination. The thorax was opened and the lungs dissected en bloc. The lungs were weighed and the trachea was cannulated with polyethylene tubing and lavaged with 5 sequential aliquots of 3 ml NS.

The volume of lavage returned was similar in all groups and always < 80% of that instilled. Lavage fluid was centrifuged at 200 g for 10 minutes. The resulting cell pellet was resuspended in 1 ml Hank's Balanced Salt Solution (HBSS) for performing cell count and differentials.

<#s> The supernatant was assayed for protein and hemoglobin, as indices of acute injury.

Visual observation of the excised lungs of the hamsters injected with HLE alone showed gross injury by hemorrhage and microscopically in intralveolar bleeding and inflammation. The latter was monitored by assessing hemoglobin, protein and polymorphonuclear leukocyte content in lung lavage fluid. The data obtained is presented in Figures 2A, 2B and 2C. As evident from the data in these Figures, GAGPS markedly protected hamster lungs from acute injury, not only preventing increase in lung weight from edema, and the increase in hemoglobin and protein in the lavage fluid, but also attenuated the influx of leukocytes into the alveolar space. Essentially total protection was obtained when GAGPS was injected within 4 hours of HLE administration. GAGPS administered 8 hours prior to HLE had a modest protective effect. Similar effects were obtained with each of the three molecular weight fractions which had effectively inhibited HLE in vitro.

Dextran sulfate also markedly protected lungs from acute lung injury in like tests.

The prolonged interval of protection after a single instillation of GAGPS indicates a long residence time within the lungs. Such was assessed by determining the rate of clearance of 3H-GAGPS from the lungs. To determine the fate of GAGPS in the pulmonary tract, certain of the hamsters were sacrificed at timed intervals after administration of 0.25 ml of NS containing 136  $\mu$ g of 3H-GAGPS ( $1.60 \times 10^6$  cpm). The lungs were weighed and lavaged as described hereinabove. A 20% homogenate by weight was made of the lungs after lavage. An aliquot of homogenate was digested with tissue solubilizer (NCS) at 50°C, and the sample was then bleached with 30% hydrogen peroxide before adding to 10 ml of Hydrofluor. The 3H content was determined by scintillation counting. Aliquots (1 ml) of each lavage fluid sample was similarly counted. Results were expressed as % of the intratracheal dose of cpm administered that was present in lung/lavage at the time of sampling. Data showing such rate is presented in Table V. After 15 minutes following instillation, 30% of the administered radioactivity had disappeared. Thereafter there was a progressive decrease in the 3H activity recovered in the lavage fluid. Ten percent of the administered radioactivity was recovered in the lavage after 8 hours. Importantly, the portion of the radioactivity resident in the lung after lavage (8%) remained stable over 24 hours, thereby indicating that 3H GAGPS rapidly pools into a long-lived lung reservoir which has a long half-life.

TABLE V  
FATE OF 3H-GAGPS ADMINISTERED INTRATRACHEALLY  
TO HAMSTER LUNGS

% of Intratracheal 3H-GAGPS Recovered

Time Lung Lavage 15 min 7.5 64

1 hr 7.8 37

4 hr 7.7 21

8 hr 9.6 10 24 hr 7.4 6.5

In the compounds of the present invention, it is required that there be present significant quantities of the anion -SO<sub>3</sub><sup>-</sup>. Whereas it is not known with certainty, it is believed that the interaction between GAGPS and HLE most likely occurs by the formation of electrostatic bonds between the negatively charged sulfate groups in the polysaccharide and positively charged guanidium groups of the arginine residues located at the surface of the highly basic enzyme. It further appears that the interaction does not influence the active center of the enzyme, but causes an indirect loss of catalytic activity.

The effects of GAGPS on HLE-mediated emphysema were examined. These studies employed the same mass ratios and regimens as outlined hereinabove in assessing acute lung injury. The animals were sacrificed eight weeks after receiving HLE with or without GAGPS. The lungs were excised and inflated fixed. The extent of emphysema was determined on coded randomized sections by quantifying the alveolar intercepts in 20 randomly selected fields for each lung. HLE administered to the animals caused prominent airspace enlargement as indicated by a decrease in the number of alveolar intercepts. These observations are depicted in Figures 3A, 3B and 3C.



Protection from emphysema was obtained when the standard preparation of GAGPS (10K Daltons) was administered at a mass ratio of 1:5 (enzyme to inhibitor) within 4 hours of the subsequent delivery of HLE. Pretreatment by 8 hours was not effective. In contrast, the 6K Dalton fraction of GAGPS preparation was not effective in protecting from HLE-mediated emphysema at any pretreatment level. However, the 19K Dalton fraction of GAGPS protected when administered up to 8 hours prior to administration of the HLE.

Thus, the 10K and particularly the 19K Dalton fractions effectively attenuated the HLE-induced emphysema.

Whereas the present compounds have not been tested in humans, it is noted that those adverse effects of heparin and Arteparon which have been noted heretofore appear to be a function of the molecular weight fraction heretofore employed. For example, Arteparon and heparin, which are administered intramuscularly or intravenously, and in relatively large dosages, comprise molecular weight fractions of greater than 100,000 Daltons. In the present invention, sulfated polysaccharides of a molecular weight of less than about 20,000 Daltons have been found effective and are preferred. Further, the present inventors have discovered that, in the instance of the pulmonary diseases prevented or mediated by the presently disclosed compounds, the elastase is present in the pulmonary tract in relatively small quantities, hence the present inventors have further found that relative small dosages of the polysulfated polysaccharides are effective to provide at least 80% or better inhibition of the elastase in the pulmonary tract. This has been found to be true at elastase to inhibitor ratios of between about 1:0.2 to 1:5.

Thusly, the exposure of the patient to relatively large quantities of the polysulfated compounds is minimized, to the point that no adverse effects in hamsters has been noted when the compounds are administered to healthy animals. When administered to animals suffering from elastase-mediated diseases of the pulmonary tract, the compounds provide marked therapeutic improvement, including full recovery from the disease without adverse side effects. Further, as noted hereinabove, the compounds of the present invention, administered by aerosolization, function extracellularly, thereby minimizing adverse conditions such as disruption of normal immunoreactivity and the like. The present compounds, present extracellularly, of relatively low molecular weight, and administered in relatively small dosages are not indicated to be adverse in humans, especially in view of the observed favorable acceptability in animal models. The long effective residence time in the host of the present compounds, along with their other noted characteristics, indicates their usefulness in the treatment of both chronic and/or acute HLE-mediated lung diseases, including emphysema, cystic fibrosis, etc.

The sulfated polysaccharides of the present invention are normal constituents of the lung matrix. It has been noted by the present inventors that the present compounds are indicated to provide a tissue based protection of elastin from HLE-mediated proteolysis. This observation points out the uniqueness of the present compounds for use in the prevention and treatment of pulmonary diseases. This specificity of the present compounds further suggests a basis for the observed desired inhibitory activity of these compounds without noted deleterious effects upon those concomitant desirable and necessary metabolic activities within the pulmonary tract parenchyma.

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## Claims

### CLAIMS:

1. A medicament for the prevention or mediation of human leucocyte elastase-mediated pulmonary disease comprising a treatment effective amount of a polysulfated polysaccharide.
2. The medicament of Claim 1 wherein said medicament is administered by aerosolization.
3. The medicament of Claim 1 wherein said polysaccharide is selected from the group comprising GAGPS, dextran sulfate, chondroitin sulfate, heparan sulfate and their derivatives.
4. The medicament of Claim 1 wherein the ratio of elastase to polysaccharide in the lung is between about 1:0.2 and about 1:5.
5. The medicament of Claim 1 wherein said polysaccharide has a molecular weight of less than about 100,000 Daltons.
6. The medicament of Claim 1 wherein said treatment effective amount is determined as a function of the quantity of human leucocyte elastase present extracellularly in the pulmonary tract.
7. The medicament of Claim 1 wherein an effective amount of said polysaccharide remains extracellular within the pulmonary tract.
8. The medicament of Claim 1 wherein said polysaccharide binds substantially with the reactive ends of the molecules of human leucocyte elastase, leaving the catalytically-active central portion of said molecules free.
9. A method for the treatment of human leucocyte elastase-mediated diseases of the pulmonary tract comprising the administration by aerosolization of a treatment effective amount of a polysulfated polysaccharide to an afflicted host.
10. The method of Claim 9 wherein said polysulfated polysaccharide is selected from the group comprising GAGPS, dextran sulfate, chondroitin sulfate, and heparan sulfate.
11. The method of Claim 9 wherein said polysulfated polysaccharide comprises dextran sulfate.
12. The method of Claim 9 wherein said polysulfated polysaccharide is administered in an amount sufficient to provide a ratio of human leucocyte elastase to polysulfated polysaccharide of between about 1:0.2 and about 1:5.
13. The method of Claim 9 wherein an effective amount of said sulfated polysaccharide remains substantially extracellular within the pulmonary tract.
14. The method of Claim 9 wherein said polysulfated polysaccharide comprises GAGPS.

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<b>(21) International Application Number:</b> PCT/US91/02299 <b>(22) International Filing Date:</b> 2 April 1991 (02.04.91) <b>(30) Priority data:</b> 505,562                      5 April 1990 (05.04.90)                      US <b>(71)(72) Applicants and Inventors:</b> KENNEDY, Thomas, P. [US/US]; Erwin Road, Durham, NC 27710 (US). HOIDAL, John, R. [US/US]; 50 North Medical Drive, Salt Lake City, UT 84132 (US). <b>(74) Agents:</b> LUEDEKA, HODGES, NEELY & GRAHAM et al.; 1030 First American Center, Knoxville, TN 37902 (US).		<b>(81) Designated States:</b> AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), LU (European patent), NL (European patent), SE (European patent).  <b>Published</b> <i>With international search report.</i>
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METHOD AND MEDICAMENT FOR PREVENTION OR  
MEDICATION OF HUMAN LEUCOCYTE ELASTASE-  
MEDIATED PULMONARY DISEASES

This invention was made with government support under Contract No. 5 R01 HL 37615 awarded by the National Institutes of Health. The government has certain rights in this invention.

5 This invention relates to methods and pharmaceutically effective compounds useful in the prevention or mediation of human leucocyte elastase-mediated pulmonary diseases.

10 Human leucocyte elastase (HLE) (3.4.21.11) is a serine proteinase stored in the azurophil granules of polymorphonuclear leucocytes. HLE is capable of hydrolyzing most connective tissue components, especially elastin. Destruction of elastin and the concomitant loss of elastic recoil in the lung parenchyma has been postulated as arising  
15 from an imbalance between elastase and its primary inhibitor, alpha-1-protease. The source of such possible imbalance is not known. The presumed role of elastase is to degrade bacterial cell wall components within the confines of the phagosome once the neutrophil has ingested a microbe.  
20 Unfortunately, elastase is often released inadvertently into the nearby extracellular milieu during the process of neutrophil activation and microbial phagocytosis. To prevent injury by free elastase, alpha-1-protease binds to free elastase and inactivates it.

25 It has been suggested in the prior art that various pulmonary diseases are elastase-mediated. Emphysema, cystic fibrosis, acute respiratory distress syndrome, bronchial pneumonia, and other similar diseases are reported to be associated with excessive proteolysis, especially, elastolysis.  
30

HLE exhibits rather unusual features when compared with other serine proteases such as trypsin, chymotrypsin and porcine pancreatic elastase. In the prior art, researchers

have commonly used the more readily available chymotrypsin or porcine pancreatic elastase in studying the mechanism of catalysis of the serine family of proteases and their interaction with inhibitors. This has led to less than definitive conclusions, such as, for example, reported equivalent degradation of purified elastin by HLE and porcine pancreatic elastase, but with HLE exhibiting a preferential decrease in degradation of elastin contained in an extracellular matrix.

Several inhibitors, of a wide variety, have been developed for serine proteases, including irreversible inhibitors which are further classified as affinity-label inhibitors and mechanism-based inhibitors; and reversible inhibitors which are further classified as tight-binding, slow binding, slow tight-binding and classical. Affinity-label inhibitors have been tested with HLE but their high reactivity makes their use problematical. Mechanism-based inhibitors include haloenal lactones, chloropyrones, isocoumarins, and ynenol lactones. No in vivo studies are known to be reported for these compounds and their potential as therapeutic agents remains unknown. Whether one uses the affinity-labels or mechanism-based inhibitors, the goal is to achieve irreversible or at least long-term inhibition of the enzyme. This is precisely the mechanism for inhibition of elastase by alpha-1-protease inhibitor. This latter inhibitor, however, suffers from its susceptibility to oxidation which renders it ineffective as an elastase inhibitor. Thus, alpha-1-protease is rendered ineffective in the pulmonary tract by reason of the oxygen metabolites ( $H_2O_2$ ), oxygen-derived free radicals (super oxide anion, hydroxyl radical), spin-altered states of oxygen (singlet oxygen), hypochlorous acid or oxidants associated with smoke inhalation (cigarette smoke).

Reversible inhibitors form a stable enzyme-inhibitor complex. Examples include peptide keto esters and peptide boronic acids. Whereas peptidyl boronic acid compounds have been found effective, in vitro, they are rapidly

reversible and actually enhance HLE-induced emphysema in animal models.

Polysulfated polysaccharides, such as Arteparon (a sulfated glycosaminoglycan derivative of chondroitin sulfate), Structum (a polydisperse mixture of chondroitin-4-sulfate and chondroitin-6-sulfate, and disodium salt extracted from the cartilage of bovine trachea), and other compounds have been suggested as inhibitors of elastase in vitro (as the term is used in a general sense). However, Arteparon has been implicated in deaths from cerebral hemorrhage, thus rendering this compound and its parent, chondroitin sulfate, seriously suspect for in vivo use. In similar manner, heparin has a small, but unfortunately serious risk of immune-mediated arterial thrombosis associated with its use so that this compound is useful for "in-hospital" therapy where its potential side effects may be carefully monitored, and therefore is not indicated as a practical outpatient pharmaceutical for elastase inhibition. Moreover, the prior art teaches that the effectiveness of polysulfated polysaccharides, such as chondroitin sulfate (including Arteparon), heparin, and sulfated dextrans and chitosans are ineffective as elastase inhibitors at the lower molecular weight fractions of these compounds. The degree of sulfation of the compound is reported to strongly affect the inhibition efficacy of the compounds. For example, it has been reported that polysulfated dextrans having a molecular weight below about 100,000 Daltons are relatively ineffective as an elastase inhibitor when the degree of sulfation is less than about 1.

Contrary to the relatively voluminous teachings of the prior art that certain sulfated polysaccharides can serve as inhibitors of elastase in vitro, to the knowledge of the present inventors, there has been no successful development of an effective medicament for pulmonary tract-associated diseases that are mediated by HLE.

The problems associated with the prior art compounds and or techniques of administration of such compounds

include such matters as noted hereinabove relating to the toxicity or undesirable side effects of certain polysulfated polysaccharides, ineffectiveness in the presence of oxidants, requirement that the compound be of relatively large  
5 molecular weight which complicates its delivery to sites within the pulmonary tract and which may contribute to its toxicity or the development of side effects. Other considerations when examining the inhibition of elastase in the pulmonary tract include solubility of the compound in aqueous  
10 media, electrolytic make-up of the compound (elastase is strongly cationic), physiological half-life, dispersibility in vivo, ability to target elastase, selectivity of reactivity, etc.

It is an object, therefore, of the present invention  
15 tion to provide a pharmaceutically acceptable medicament for HLE-mediated pulmonary diseases. It is another object to provide such a medicament which may be administered by aerosolization. It is another object to provide a novel method for the treatment or prevention of elastase-mediated  
20 pulmonary diseases.

The present invention comprises a medicament for the prevention or medication of human leucocyte elastase-mediated pulmonary disease comprising a treatment effective amount of a polysulfated polysacchride.

25 In accordance with the present invention, the present inventors have discovered that through the oral application of pharmaceutically effective quantities of polysulfated polysaccharides (PS), as by the inhalation of a powder or liquid mist containing the compound, such PS  
30 effectively inhibit HLE-mediated diseases associated with the pulmonary tract. The pharmaceutically effective quantity is selected to be in the range of about 10 mg to about 100 mg q6h of the PS, based on the inhibitory activity of the medicament and the load of HLE in the lungs. The discovery  
35 that such relatively low dosages of the PS are effective permits the compound to be administered by aerosol, as opposed to intramuscular or intravenous administration which



are recognized as potentially giving rise to adverse effects in vivo. Further, such discovery has provided the means for limiting the exposure of the body to the potentially damaging side effects of certain of the PS compounds which now appear to be partially a consequence of, or at least exacerbated by, the prior art requirement that such compounds be administered in substantially sized dosages in order for them to be effective. Still further, the PS of the present invention have been found to effectively seek out the alveolar interstices and remain available for half-life time periods that permit the administration of the compound in amounts and at times that are reasonable and acceptable for patients. To this end, the present compounds have been found effective when administered orally as by inhalation at intervals of up to about eight hours. The sulfated polysaccharides of the present invention are "naturally occurring" and commonly are present in the extracellular matrix. The fact that sulfated polysaccharides of the present invention are normal constituents of lung matrix indicates that these compounds provide a tissue based protection of elastin from HLE-mediated proteolysis, thereby differentiating the present compounds from other compounds known to provide in vivo inhibition of elastolytic activity.

The compounds of the present invention are reversible as regards their inhibitory effect upon elastase. That is, the elastase is inhibited as by the electrostatic binding of the PS to the opposite ends of the elastase molecule, leaving the effective center portion of the elastase molecule unaltered. Importantly, the compounds of the present invention remain extracellular so that they are available to inhibit extracellular elastase without disruption of the desirable and beneficial function of intracellular elastase, which in its "natural" and effective state is a host defense against bacteria. Further, the PS compounds of the present invention are not subject to being rendered ineffective by oxidants in the pulmonary tract, they are not adversely affected by normal pH fluctuations in the tract, and they

are selective in that they do not attack the parenchyma tissue nor inhibit the normal functioning of other desirable metabolic activities, including desirable proteolytic activities. The present PS compounds have been found to not  
5 induce adverse hemorrhaging, and in fact have been noted to effect a reduction in established hemorrhaging due to elastase-induced diseases in the pulmonary tract.

The present PS compounds, when administered in accordance with the present disclosure are considered effective in the treatment of any HLE-mediated disease of the  
10 pulmonary tract. For example, it is known that the bacteria, Pseudomonas aeruginosa, is commonly present in the pulmonary passages of normal persons but such persons do not contract pneumonia. However, up to two percent of the  
15 patients entering hospitals contract pneumonia and of these patients, 50% die from such hospital-contacted pneumonia. The present PS compounds, inhaled routinely in the course of a hospital stay, are deemed to provide prophylactic protection against such pneumonia, most likely by reason of the  
20 fact that the present PS compounds inhibit the development of colonization sites for the bacteria by the proteolytic activity of elastase in the pulmonary tract.

FIGURE 1 is a graphical representation of data indicating the inhibition of HLE by sulfated polysaccharides. The substrate used was suc-ala<sub>2</sub>-val-pNA.  
25

FIGURES 2A, 2B and 2C are graphical representations of data showing the effect of GAGPS on acute lung injury induced by HLE. FIGURE 2A depicts the hemoglobin/ml lavage, FIGURE 2B depicts the protein/ml lavage, and FIGURE  
30 2C depicts the granulocyte count/ml lavage.

FIGURES 3A, 3B and 3C are graphical representations depicting the effect of GAGPS on HLE-mediated emphysema as indicated by quantification of the alveolar intercepts in randomly selected fields for each lung examined. In  
35 FIGURE 3A, 6K GAGPS was administered four hours after the HLE; in FIGURE 3B, 10K GAGPS was administered simultaneously with the HLE; and, in FIGURE 3C, 19K GAGPS was administered

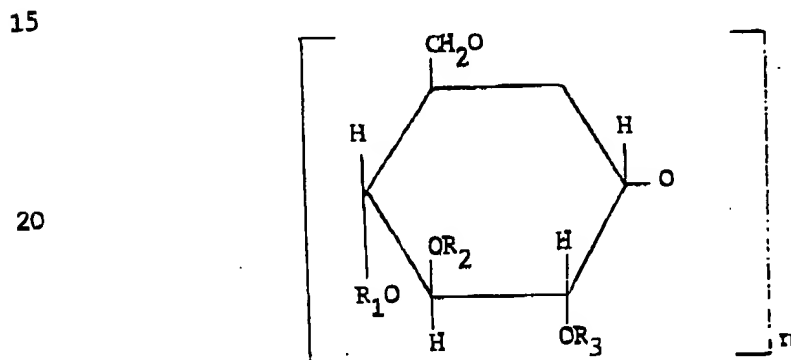
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four hours after the HLE. In all instances, the extent of emphysema was reduced after addition of the GAGPS and remained reduced for up to eight hours.

FIGURE 4 is a graphical representation depicting the effect of dextran sulfate on acute lung injury induced by HLE as represented by the analysis of protein in lavage.

FIGURE 5 is a graphical representation depicting the effect of dextran sulfate on acute lung injury induced by HLE as represented by the analysis of hemoglobin in lavage.

In accordance with the present invention, the preferred inhibitor is dextran sulfate having the following formula:



where:

$n = \text{SO}_3^-, \text{H}$

$\text{R}_1, \text{R}_2$  and  $\text{R}_3$  each = a lower alkyl

This compound is a derivative of dextran, or polyglucose composed of alpha-D-glucopyranosyl units linked predominantly alpha-D(1-6) and differing only in chain length as defined in The Merck Index, 10th Ed., 1923; 426. Dextran is produced by bacteria growing on a sucrose substrate. A number of methods of production exist, including methods of removing pyrogens, and for producing clinical

dextran (U.S. Patent Nos. 2,762,727 and 2,972,567). Dextran sulfate is prepared by boiling dextran with sulfuric acid to reduce the molecular weight and esterifying the degraded dextran with chlorosulfonic acid in pyridine as described in  
 5 U.S. Patent No. 2,715,091, which patent is incorporated herein by reference.

The activity of dextran sulfate was tested by monitoring the ability of HLE purified from an extract of polymorphonuclear leukocytes using Matrex Gel Orange A  
 10 chromatography followed by cation exchange chromatography on Bio-Rex 70 as described by Kao et al., Proteinase 3: A Distinct Human Polymorphonuclear Leukocyte Proteinase That Produces Emphysema in Hamsters, J. Clin Invest 1988; 82:1963-1973 to cleave the synthetic elastase substrate  
 15 succinyl-tri-alanyl-p-nitroanilide, causing an increase in spectrophotometric optical density at 405 nm. The reaction was run in 0.125 M HEPES buffer (pH 7.5) containing 0.125% Triton X-100, 3 mM substrate, 20 µg/ml of HLE and various  
 20 molar ratios of elastase to inhibitor. Various molecular weight fractions of dextran sulfate were tested, the results being depicted in Table I.

TABLE I  
 EFFECT OF DEXTRAN SULFATE  
 AND DEXTRAN ON HLE

25	Ratio of Elastase to Inhibitor	O.D. Change at 405 nm	% Inhibition
	A. Dextran Sulfate (mol. wt. 5000)		
	1:0	0.085	0
30	1:0.02	0.081	5
	1:0.2	0.066	29
	1:0.4	0.045	47
	1:0.5	0.040	53
	1:1	0.014	84
35	1:2	0.010	88
	1:4	0.004	95

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B. Dextran Sulfate  
(mol. wt. 8000)

	1:0	0.085	0
	1:0.02	0.068	20
5	1:0.2	0.062	27
	1:0.4	0.043	49
	1:0.5	0.032	62
	1:1	0.011	77
	1:2	0.007	82
10	1:4	0.004	95

C. Dextran  
(mol. wt. 9000)

	1:0	0.099	0
	1:0.1	0.099	0
15	1:0.2	0.099	0
	1:0.4	0.113	0
	1:0.5	0.111	0
	1:1	0.112	0
	1:2	0.112	0

20 As shown in Table I, dextran alone provides no inhibitory activity toward HLE. On the other hand, the inhibitory activity of dextran sulfate is almost complete at a ratio of 1:2 elastase to inhibitor. Such activity is thus a function of the molecular weight of the dextran sulfate and its degree of sulfation.

25 Chondroitin sulfate having a molecular weight of 55,000 Daltons (purchased from Sigma Chemical of St. Louis, MO.) and heparan sulfate having a molecular weight of 40,000 Daltons purified from EHS sarcoma as described by Hassell et al., Isolation of a Heparan-sulfate Contain-  
30 ing Proteoglycan From Basement Membrane, Proc. Natl. Acad. Sci. USA, 1980; 77:4494-4498, were tested for inhibitory activity in like manner to the testing of dextran sulfate. The results of such tests are given in Tables II and III.

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TABLE II  
EFFECT OF CHONDROITIN SULFATE  
ON HLE

	Ratio of Elastase to Inhibitor	O.D. Change at 405 nm	% Inhibition
5	1:0	0.080	0
	1:0.1	0.018	77
	1:0.2	0.014	82
	1:0.4	0.014	82
	1:0.5	0.015	81
10	1:1	0.015	81

TABLE III  
EFFECT OF HEPARAN SULFATE  
ON HLE

	Ratio of Elastase to Inhibitor	O.D. Change at 405 nm	% Inhibition
15	1.0	0.096	0
	1:0.1	0.083	14
	1:0.2	0.052	46
	1:0.4	0.029	70
	1:0.5	0.024	75
20	1:1	0.017	82

Both chondroitin sulfate and heparan sulfate inhibited elastase in vitro almost completely at a 1:1 ratio of inhibitor to elastase.

In further testing, the activity of HLE was monitored using the specific synthetic chromogenic substrate suc-ala<sub>2</sub>-val-pNA. The method used is described by Barrett, A. J., Cathepsin G, Methods Enzymol. 1981; 80: 561-565 with modifications. The assay mixture of 1 ml

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contained 0.3 mM substrate (100  $\mu$ l 3 mM in DMSO) in 50 mM HEPES buffer, pH 7.5. The reaction was started by addition of 100  $\mu$ l of HLE (20  $\mu$ g/ml). Activity against the substrate was determined by release of 4-nitroaniline as indicated by an increase in OD at 405 nm over 3 minutes. Inhibition was assessed by preincubation of HLE with sulfated polysaccharides for 30 min at 37°C prior to initiating the reaction. Tests were performed employing dextran sulfate, heparin, heparan sulfate, chondroitin sulfate, dextran sulfate and dextran. The results of these tests are presented in Figure 1 wherein it is noted that each of these additional compounds in a concentration of less than 400 ng/ml is an effective inhibitor of HLE.

The activity of HLE itself was also assessed with insoluble elastin as the substrate. Bovine ligament elastin was prepared by the method of Starcher and Galione (Starcher *et al*, Purification and Comparison of Elastins from Different Animal Species, Anal Biochem 1976; 74:411-447) and assessed for purity by amino acid analysis. Its degradation was assayed using elastin radiolabelled with [ $^3$ H] NaBH $_4$  following the methods described by Stone *et al*, Proteolysis of Insoluble Elastin, Methods Enzymol 1982: 82:588-605. The tritiated powdered elastin was homogenized and washed in PBS, pH 7.4. The reaction mixture containing the reference enzyme or sample preincubated with inhibitor was added to a 5 mg aliquot of  $^3$ H-elastin and incubated at 37°C, pH 7.4. Solubilized peptides were separated from the elastin suspension by filtration through medium porosity filter paper. The rate of degradation was determined by quantifying the  $^3$ H-peptides solubilized. Table IV presents the results of these tests and indicates that GAGPS of molecular weights between about 6,000 and 19,000 Daltons effectively reduces the HLE activity at molar ratios of HLE to GAGPS as low as about 1:0.2 at the higher molecular weights while requiring higher ratios, i.e. above about 1:0.5 at the lower molecular weights.

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TABLE IV  
HLE ACTIVITY AGAINST  
<sup>3</sup>H-ELASTIN IN PRESENCE OF GAGPS

5	Molar Ratios HLE:GAGPS	GAGPS			
		2.8K	6K	10K	19K
10	1:0	100	100	100	100
	1:0.1	136	113	87	58
	1:0.2	95	84	48	17
	1:0.4	127	81	19	31
	1:0.5	106	56	-	14
	1:1	118	25	-	18

The ability of polysulfated polysaccharides to prevent HLE-mediated acute lung injury was assessed in female Syrian golden hamsters (Harlan Industries, Indianapolis, IN.) weighing 90-110 g. Pentobarbital anesthetized hamsters were injected intratracheally with 0.25 ml sterile 0.9% saline (NS) or 0.25 ml NS containing fractions of Arteparon (GAGPS), a supersulfated derivative of chondroitin sulfate, having respective molecular weights of 2,800, 10,000 and 19,000 Daltons and with a limited degree of polydiversity. These injections of GAGPS were followed at timed intervals by injection of HLE in 0.25 ml NS. Twenty-four hours after the treatment, anesthetized animals were sacrificed by exsanguination. The thorax was opened and the lungs dissected en bloc. The lungs were weighed and the trachea was cannulated with polyethylene tubing and lavaged with 5 sequential aliquots of 3 ml NS. The volume of lavage returned was similar in all groups and always  $\leq$  80% of that instilled. Lavage fluid was centrifuged at 200 g for 10 minutes. The resulting cell pellet was resuspended in 1 ml Hank's Balanced Salt Solu-



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tion (HBSS) for performing cell count and differentials. The supernatant was assayed for protein and hemoglobin, as indices of acute injury.

Visual observation of the excised lungs of the hamsters injected with HLE alone showed gross injury by hemorrhage and microscopically in intralveolar bleeding and inflammation. The latter was monitored by assessing hemoglobin, protein and polymorphonuclear leukocyte content in lung lavage fluid. The data obtained is presented in Figures 2A, 2B and 2C. As evident from the data in these Figures, GAGPS markedly protected hamster lungs from acute injury, not only preventing increase in lung weight from edema, and the increase in hemoglobin and protein in the lavage fluid, but also attenuated the influx of leukocytes into the alveolar space. Essentially total protection was obtained when GAGPS was injected within 4 hours of HLE administration. GAGPS administered 8 hours prior to HLE had a modest protective effect. Similar effects were obtained with each of the three molecular weight fractions which had effectively inhibited HLE *in vitro*. Dextran sulfate also markedly protected lungs from acute lung injury in like tests.

The prolonged interval of protection after a single instillation of GAGPS indicates a long residence time within the lungs. Such was assessed by determining the rate of clearance of  $^3\text{H}$ -GAGPS from the lungs. To determine the fate of GAGPS in the pulmonary tract, certain of the hamsters were sacrificed at timed intervals after administration of 0.25 ml of NS containing 136  $\mu\text{g}$  of  $^3\text{H}$ -GAGPS ( $1.60 \times 10^6$  cpm). The lungs were weighed and lavaged as described hereinabove. A 20% homogenate by weight was made of the lungs after lavage. An aliquot of homogenate was digested with tissue solubilizer (NCS) at  $50^\circ\text{C}$ , and the sample was then bleached with 30% hydrogen peroxide before adding to 10 ml of Hydrofluor. The  $^3\text{H}$  content was determined by scintillation counting. Aliquots (1 ml) of each lavage fluid sample was similarly

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counted. Results were expressed as % of the intratracheal dose of cmp administered that was present in lung/lavage at the time of sampling. Data showing such rate is presented in Table V. After 15 minutes following instillation, 30% of the administered radioactivity had disappeared. Thereafter there was a progressive decrease in the  $^3\text{H}$  activity recovered in the lavage fluid. Ten percent of the administered radioactivity was recovered in the lavage after 8 hours. Importantly, the portion of the radioactivity resident in the lung after lavage (8%) remained stable over 24 hours, thereby indicating that  $^3\text{H}$ -GAGPS rapidly pools into a long-lived lung reservoir which has a long half-life.

TABLE V  
FATE OF  $^3\text{H}$ -GAGPS ADMINISTERED INTRATRACHEALLY  
TO HAMSTER LUNGS

% of Intratracheal $^3\text{H}$ -GAGPS Recovered			
	Time	Lung	Lavage
	15 min	7.5	64
20	1 hr	7.8	37
	4 hr	7.7	21
	8 hr	9.6	10
	24 hr	7.4	6.5

In the compounds of the present invention, it is required that there be present significant quantities of the anion  $-\text{SO}_3^-$ . Whereas it is not known with certainty, it is believed that the interaction between GAGPS and HLE most likely occurs by the formation of electrostatic bonds between the negatively charged sulfate groups in the polysaccharide and positively charged guanidium groups of the arginine residues located at the surface of the highly basic enzyme. It further appears that the interaction does not influence the active center of the enzyme, but causes an indirect loss of catalytic activity.

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The effects of GAGPS on HLE-mediated emphysema were examined. These studies employed the same mass ratios and regimens as outlined hereinabove in assessing acute lung injury. The animals were sacrificed eight weeks after receiving HLE with or without GAGPS. The lungs were excised and inflation fixed. The extent of emphysema was determined on coded randomized sections by quantifying the alveolar intercepts in 20 randomly selected fields for each lung. HLE administered to the animals caused prominent airspace enlargement as indicated by a decrease in the number of alveolar intercepts. These observations are depicted in Figures 3A, 3B and 3C. Protection from emphysema was obtained when the standard preparation of GAGPS (10K Daltons) was administered at a mass ratio of 1:5 (enzyme to inhibitor) within 4 hours of the subsequent delivery of HLE. Pretreatment by 8 hours was not effective. In contrast, the 6K Dalton fraction of GAGPS preparation was not effective in protecting from HLE-mediated emphysema at any pretreatment level. However, the 19K Dalton fraction of GAGPS protected when administered up to 8 hours prior to administration of the HLE. Thus, the 10K and particularly the 19K Dalton fractions effectively attenuated the HLE-induced emphysema.

Whereas the present compounds have not been tested in humans, it is noted that those adverse effects of heparin and Arteparon which have been noted heretofore appear to be a function of the molecular weight fraction heretofore employed. For example, Arteparon and heparin, which are administered intramuscularly or intravenously, and in relatively large dosages, comprise molecular weight fractions of greater than 100,000 Daltons. In the present invention, sulfated polysaccharides of a molecular weight of less than about 20,000 Daltons have been found effective and are preferred. Further, the present inventors have discovered that, in the instance of the pulmonary diseases prevented or mediated by the presently disclosed compounds, the elastase is present in the pulmonary tract

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in relatively small quantities, hence the present inventors have further found that relative small dosages of the polysulfated polysaccharides are effective to provide at least 80% or better inhibition of the elastase in the pulmonary tract. This has been found to be true at elastase to inhibitor ratios of between about 1:0.2 to 1:5. Thusly, the exposure of the patient to relatively large quantities of the polysulfated compounds is minimized, to the point that no adverse effects in hamsters has been noted when the compounds are administered to healthy animals. When administered to animals suffering from elastase-mediated diseases of the pulmonary tract, the compounds provide marked therapeutic improvement, including full recovery from the disease without adverse side effects. Further, as noted hereinabove, the compounds of the present invention, administered by aerosolization, function extracellularly, thereby minimizing adverse conditions such as disruption of normal immunoreactivity and the like. The present compounds, present extracellularly, of relatively low molecular weight, and administered in relatively small dosages are not indicated to be adverse in humans, especially in view of the observed favorable acceptability in animal models. The long effective residence time in the host of the present compounds, along with their other noted characteristics, indicates their usefulness in the treatment of both chronic and/or acute HLE-mediated lung diseases, including emphysema, cystic fibrosis, etc.

The sulfated polysaccharides of the present invention are normal constituents of the lung matrix. It has been noted by the present inventors that the present compounds are indicated to provide a tissue based protection of elastin from HLE-mediated proteolysis. This observation points out the uniqueness of the present compounds for use in the prevention and treatment of pulmonary diseases. This specificity of the present compounds further suggests a basis for the observed de-

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sired inhibitory activity of these compounds without noted deleterious effects upon those concomitant desirable and necessary metabolic activities within the pulmonary tract parenchyma.

CLAIMS:

1. A medicament for the prevention or mediation of human leucocyte elastase-mediated pulmonary disease comprising a treatment effective amount of a polysulfated polysacchride.

2. The medicament of Claim 1 wherein said medicament is administered by aerosolization.

3. The medicament of Claim 1 wherein said polysacchride is selected from the group comprising GAGPS, dextran sulfate, chrondroitin sulfate, heparan sulfate and their derivatives.

4. The medicament of Claim 1 wherein the ratio of elastase to polysaccharide in the lung is between about 1:0.2 and about 1:5.

5. The medicament of Claim 1 wherein said polysaccharide has a molecular weight of less than about 100,000 Daltons.

6. The medicament of Claim 1 wherein said treatment effective amount is determined as a function of the quantity of human leucocyte elastase present extracellularly in the pulmonary tract.

7. The medicament of Claim 1 wherein an effective amount of said polysaccharide remains extracellular within the pulmonary tract.

8. The medicament of Claim 1 wherein said polysaccharide binds substantially with the reactive ends of the molecules of human leucocyte elastase, leaving the catalytically-active central portion of said molecules free.

9. A method for the treatment of human leucocyte elastase-mediated diseases of the pulmonary tract comprising the administration by aerosolization of a treatment effective amount of a polysulfated polysaccharide to an afflicted host.

10. The method of Claim 9 wherein said polysulfated polysaccharide is selected from the group comprising GAGPS, dextran sulfate, chondroitin sulfate, and heparan sulfate.

11. The method of Claim 9 wherein said polysulfated polysacchride comprises dextran sulfate.

12. The method of Claim 9 wherein said polysulfated polysacchride is administered in an amount sufficient to provide a ratio of human leucocyte elastase to polysulfated polysacchride of between about 1:0.2 and 5 about 1:5.

13. The method of Claim 9 wherein an effective amount of said sulfated polysaccharide remains substantially extracellular within the pulmonary tract.

14. The method of Claim 9 wherein said polysulfated polysaccharide comprises GAGPS.

# INTERNATIONAL SEARCH REPORT

International Application No. **PCT/US91/02299**

<b>I. CLASSIFICATION</b>		<b>SUBJECT MATTER</b> (If several classification symbols apply, indicate all) *	
According to International Patent Classification (IPC) or to both National Classification and IPC			
IPC(S): A61K 31/725, 31/715; C12N 9/64, 9/66			
US, CL. 514/54, 56, 59; 435/218, 219, 226			
<b>II. FIELDS SEARCHED</b>			
Minimum Documentation Searched ?			
Classification System	Classification Symbols		
CL. U.S.	514/54, 56, 59; 435/218, 219, 226		
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched *			
Chemical Abstracts Service: heparan sulfate or heparin or dextran sulfate or chondroitin sulfate or sulfate# with polysaccharide# and elastase or serine protease and pulmon? or emphysema or cystic fibrosis or respiratory distress			
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> * Syndrome			
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages **		Relevant to Claim No. **
A	US, A, 4,496,689 (MITRA) 29 January 1985.		1-14
A	US, A, 4,686,288 (LORMEAU ET AL) 11 August 1987.		1-14
A	US, A, 4,725,545 (POWERS) 16 February 1988.		1-14
X	US, A, 4,808,570 (MICHAELI) 28 February 1989, see entire document.		1-8
X	US, A, 4,840,941 (UENO ET AL) 20 JUNE 1989, see entire document.		1-8
Y	US, A, 4,847,202 (POWERS) 11 July 1989, see entire document.		9-14
A	SU, A, 731,967 (KIEV TUBERCULOS) 12 May 1980.		1-14
A	EP, A, 0,130,550 (BRAUN MELSUNGENAG) 09 January 1985.		1-14
<p>* Special categories of cited documents: **</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p>			
<b>IV. CERTIFICATION</b>			
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report	
22 June 1991		23 JUL 1991	
International Searching Authority		Signature of Authorized Officer	
ISA/US		Nancy S. Carson	

Form PCT/ISA/210 (second sheet) (Rev. 11-87)



III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)			
Category	Citation of Document	with indication, where appropriate, of the relevant passages	Relevant to Claim in
Y	BIOCHEMICAL PHARMACOLOGY. Vol. 29. issued 1980, Baici et al. "Inhibition of human elastase from poly morphonuclear leucocytes by 2-glycosaminoglycan polysulfate (Arteparon <sup>®</sup> )", pages 1723-1727. see entire document.		9-14
Y	EUROPEAN JOURNAL OF BIOCHEMISTRY. Vol. 137. issued 1983. Peterson. "Modulatory effects on proteinase kinetics caused by association of both enzyme and substrate to heparin", pages 531-535. see entire document.		9-14
X	"Windholz et al., "THE MERCK INDEX". (tenth edition) published 1983, by Merck & Co., Inc. (N.J.). pages 314. 315, 427, 672, 673. see entries 2194. 2915 and 4543.		1-8
Y	BIOCHEMICAL JOURNAL. Vol. 252 issued 01 June 1988, Redini et al. "Inhibitions of leucocyte elastase by heparin and its derivatives", pages 515-519. see entire document.		9-14
Y	BIOCHEMICAL PHARMACOLOGY. Vol. 37. no. 22, issued 15 November 1988. Redini et al. "Influence of heparin fragments on the biological activities of elastase(s) and $\alpha_1$ -proteinase inhibitor." pages 4257-4261. see entire document.		9-14
A	AMERICAN REVIEW OF RESPIRATORY DISEASES. Vol. 131. issued 1985. Vered et al. "A new elastase inhibitor from <u>Streptococcus Pneumoniae</u> protects against acute lung injury induced by neutrophil granules", pages 131-133.		1-14
A	EXPERIMENTAL LUNG RESEARCH, Vol. 14, issued 1988, Vered et al. "Inhibition of human neutrophil elastase by bacterial polyanions", pages 67-83.		1-14
A	THE JOURNAL OF CLINICAL INVESTIGATION Vol. 84, issued October 1989. Hubbard et al. "Recombinant DNA-produced $\alpha_1$ - 1-antitrypsin administered by aerosol augments lower respiratory tract antineutrophil elastase defenses in individuals with $\alpha_1$ -antitrypsin deficiency"; pages 1349-1354.		1-14